Synopsis of the thesis entitled

CHEMICAL SPECIATION OF L-PROLINE AND L-VALINE COMPLEXES WITH SOME ESSENTIAL METAL IONS IN LOW DIELECTRIC MEDIA

By

P. BHUSHANAVATHI, M.Sc., M.Phil.,

(To be submitted to the Andhra University in partial fulfillment for the award of the Degree of Doctor of Philosophy)

Under the guidance of

Prof. G. NAGESWARA RAO

Department of Inorganic and Analytical Chemistry,

Andhra University, Visakhapatnam-530003,

INDIA.

SYNOPSIS

The role of essential trace metal ions in biological systems is well recognized in recent years. Trace elements including the heavy metals play a very important role in a living organism. They serve as biostimulaters, catalysts and co-factors and form indispensable structural components of a number of macro-molecules. This is supported by the fact that several trace elements occur in living systems in definite proportions. If they are deficient or absent altogether, the organism fails to grow normally. However, the trace metals which are essential for growth of an organism may become harmful if their concentration is raised a little. There is a thin line demarcating the deficient, sufficient and the condition of excess supply. One goal of modern chemical research is to elucidate in detail the roles of the essential trace elements.

The toxic action of most of the trace metals stems from the fact that they are capable of forming strong bonds with metabolically active groups within a living system. Under normal conditions these bonds serve to provide important linkages which keep an organic molecule in proper configuration to perform some specific function. But under conditions of an over-dose of the metal these bonds are formed at random and cause symptoms of toxicity. Studies of the interaction of the essential trace metal ions with the bioligands and others as models in vitro studies throw light on the mechanism of

adverse effects of the metal ions in metabolic processes and also in discovering antidotes.

Due to difficulties in investigating the complex biological macromolecules in the presence of a variety of chemical compounds, use of model compounds which have similar characteristics is of vital importance. The model compounds should be comparatively more soluble and amenable for spectroscopic and electrometric studies than biomolecules, yielding unambiguous information regarding structural details and reaction mechanism.

These studies involve the determination of different forms of the trace metal ions and their speciation. Speciation profoundly influences both the toxicity and bioavailability of an element. The speciation studies of essential metal ion complexes are useful to identify those metal species that are likely to have adverse effect on biota. Better computing and analytical methods led to an understanding of the speciation underlying many chemical reactions, with interesting conclusions, in industry, medicine and in environmental studies.

Some of the trace metal ions like cobalt, nickel and copper are essential and any variation in their concentrations leads to metabolic disorders. Cobalt is associated with cyanocobalamin, a life saving vitamin. Deficiency of cobalt in human body leads to pernicious anaemia, but cobalt is mildly toxic and it depresses the thyroid activity and high cobalt diet may cause goitre due to decrease in

iodine level. Nickel is present in the enzymes like urease, which is required in nucleic acid and lipid metabolism and iron absorption. Deficiency of nickel causes depressed growth and impaired iron absorption. The toxic symptoms of nickel are dermatitis, nasal and lung cancers. Copper is an essential trace nutrient to all high plant and animal life. In humans, it is found primarily in the blood stream, as a co-factor in various enzymes and in copper based pigments. Liver plays a central role in copper metabolism. It sequesters and stores copper, converts it into a bile component and produces some of copper proteins including ceruloplasmin. However higher amounts of copper can be poisonous and even fatal to organisms.

Good drugs and powerful ligands have an important feature in common i.e. they possess electron donor groups. So it follows that reliable speciation knowledge can be employed to optimize drug efficiency to minimize side reactions and to predict undesirable clinical side effects.

Proline is non essential imino acid containing pyrrole type nitrogen rather than the amino nitrogen of the amino acids. The distinctive cyclic structure of proline's side chain gives proline an exceptional conformational rigidity compared to other amino acids. Proline is responsible for collagen formation, arteriosclerosis prevention, blood pressure maintenance, and tissue repair in human body. It helps to break down proteins for use in creating new, healthy muscle cells.

Valine is an essential amino acid and important for muscle metabolism and the repair/growth of tissue. For muscle growth valine promotes protein production. It also maintains nitrogen balance in human body. Because of the branched chain it can be used as an energy source in muscles instead of glucose. It may be helpful in treating liver and gallbladder disease. Both proline and valine are bidentate ligands with high affinity to metals; hence, the author selected these ligands to study the effect of imino group and cyclic side chain of proline on the stability of complexes with metal ions.

Due to the above facts there is every likelihood for Co(II), Ni(II) and Cu(II) and proline and valine to interact in the cells. Hence the author studied the chemical speciation of Co(II), Ni(II) and Cu(II) with proline and valine in vitro, under mimicked physiological conditions.

Since many reactions of biological importance take place in solution phase, measurements in anhydrous media are of unusual interest in understanding the role of the solvent. A recent application of speciation studies was proposed for estimating an equivalent solution dielectric constant for the active site cavity. This brought a renaissance in the study of complex equilibria in aqua-organic mixtures. In the present study 1, 2-propanediol (PG) and acetonitrile (AN) solvents are chosen to mimic the polarity of the active sites. PG is an amphiprotic and coordinating solvent. It is a structure former and enhances the water structure in PG-water mixtures. Hence it removes water from the coordination sphere of metal ions, making them more

reactive towards the ligands. AN is a protophobic, dipolar aprotic and coordinating solvent. It is a structure breaker of water and disrupts the water structure to form AN-water complex of the formula $AN.H_2O$. Hence, these media are chosen to study the effect of cosolvent on the complex equilibria of proline and valine with metal ions, Co(II), Ni(II) and Cu(II) to mimic the physiological conditions.

The studies carried out under the present experimental conditions are useful to understand (i) the role played by the active site cavities in the biological molecules, (ii) the type of complex formed by the metal ions, and (iii) the bonding behavior of the protein residues with metal ions. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of amino acids and their metal complexes in the biofluids.

The author has investigated the protonation equilibria of proline and valine and their binary and ternary complexes with Co(II), Ni(II) and Cu(II) in PG- and AN-water mixtures of varying compositions. The computer augmented modeling studies are carried out to arrive at the best fit chemical models and the models are validated by statistical treatment of the data. The literature systems and their stability constants have been searched from the computer databases MLHAQ (stability constants in aqueous medium) and BAQOR (betas in aqua-organic media) which are developed in this laboratory. The species are detected and confirmed from the experimental conditions, formation

curves, simulation curves and by the retrieval of protonation constants from metal-ligand titration data. The results obtained are presented in this thesis which is organized into five chapters and each chapter divided into sections. The references cited in the text are given at the end of the respective chapters.

In **Chapter 1**, the author describes the salient features of the amino acid metabolism and the role played by proline and valine in the physiological systems. Various biological aspects of the metal ions like absorption, storage, transport and excretion have been presented. The nature and importance of the solvents (PG and AN) are discussed. The significance of the chemical speciation and proposed study are also included in this chapter.

Chapter 2 contains the experimental details for chemical speciation of the title complexes and the modeling strategies adopted in the present investigation. Preparation and standardization of solutions, equilibration and calibration of electrodes and pH metric titration procedures are described in detail. Preprocessing techniques of data like the Gran plot method, SCPHD and modeling strategy employed for arriving at the best fit chemical models are discussed. The validity of these models is checked by statistical treatment of the data. The need for the simulation of experimental data by the program SCPHD and the role of these data in validating the best fit model is outlined. An overview of the computer program MINIQUAD75 is also presented.

In **Chapter 3**, the results of protonation-deprotonation equilibria of proline and valine is presented. The effects of systematic errors and dielectric constant of solvent-water media on the protonation constants and the distribution of protonated and deprotonated species is presented.

In **Chapter 4**, the effect of cosolvent (PG and AN) on the stability of binary metal complexes of proline and valine with Co(II), Ni(II) and Cu(II) is studied. It includes detection of protonated species, the results of electrometric studies on the metal-ligand systems, retrieval of protonation constants, perturbation in stability constants due to systematic errors in the concentrations of ingredients and trends observed in the stabilities of various complex species with the variations in the relative concentrations of metal, ligand and solvent are discussed. The relative distribution of the species with respect to pH is also included.

In **Chapter 5**, the results of formation of mixed ligand complexes of Co(II), Ni(II) and Cu(II) with proline and valine in PG- and AN-water mixtures are discussed in detail. The existence of ternary species is established from modeling studies. The relative concentrations and stabilities of the ternary species are compared with those of the binary species. The extra stability associated with the ternary complexes is attributed to the interactions outside the coordination sphere, formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect, stacking

interactions and electrostatic interactions between non-coordinated charge groups of the ligands.

The conclusions section summarizes the salient findings of the present investigations.

1. Proline has one dissociable carboxyl proton and one imino group which can associate with a proton. It exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^-, successively, with increase in pH.
2. Valine has one dissociable carboxyl proton and one amino group which can associate with a proton. It exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^-, successively, with increase in pH.
3. Most of the literature studies reported ML^+ and ML_2 species for proline and valine. But the present study reports a protonated species (MLH^{2+}) in addition to the unprotonated species. Protonated complexes are formed at lower pH and unprotonated complexes at higher pH in both PG- and AN-water media.
4. The interaction of metal ions with proline and valine infers the formation of ternary complex species MLX, $MLXH^+$ and $MLXH_2^{2+}$ for Co(II) and Ni(II) and Cu(II) in PG-water and AN-water media.